Original Research Article

Clinical and microbiological implications of time to positivity of blood cultures in adult patients with blood stream infections

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ABSTRACT

Background: Blood stream infections (BSI) are one of the serious and life threatening complications associated with high morbidity and mortality. Identification of patients without bacterial infections is an important component of antimicrobial stewardship. With the advances in the automated blood culture systems especially with the continuous monitoring systems, time to positivity (TTP) of blood cultures has been reduced drastically thereby allowing faster de-escalation of the antibiotics.

Aim and Objective: In this study, we have analysed the TTP of different bacterial isolates, and the effects of initiation of antimicrobials and blood volume on TTP.

Materials and Methods: Adult patients with monomicrobial bacteraemia in an academic hospital were included retrospectively over a four-year period. Time to positivity was recorded for each positive sample. Information about the timing of blood sample collection i.e. before or after start of antibiotics was collected from the blood culture requisition form. The blood volume in the blood culture bottle is the virtual blood volume given by the Bact-Alert Virtuo® instrument.

Results: A total of 38,606 blood culture samples that flagged positive from adult patients with suspected BSIs were included. 79% of the samples had a TTP of less than 24 hours and 15% of the samples had TTP of 24 to 48 hrs. Only 6% of the samples had TTP of more than 48 hours. Gram negative pathogens (Average TTP- 12.5hours) have shorter TTP when compared to Gram positive pathogens (Average TTP- 15.4 hours).

Conclusion: With the use of modern automated blood culture systems, TTP can be used as a tool to guide the antimicrobial therapy and early de-escalation of the empirical antibiotics thereby reducing the emergence of antimicrobial resistance.

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1. Introduction

The prompt detection of bacteraemia is crucial, requiring both the prompt initiation of broad-spectrum antimicrobial agents and the timely collection of blood cultures. Delays in these actions can lead to negative outcomes, including the possibility of death. When assessing bacteraemia, healthcare professionals often depend on blood culture outcomes to discontinue antibiotic treatment. Consequently, comprehending the time required for blood cultures to exhibit positive results for specific organisms and within specific medical specialties becomes a crucial aspect in the effort to reduce overall antimicrobial duration. [1]

The term "time-to-positivity" (TTP) in blood cultures refers to the duration between the initiation of incubation and the detection of a positive signal. Over time, it has

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been acknowledged as a valuable indicator in patient care, with its significance gradually unveiled to different extents.[2] TTP has been suggested as both a diagnostic and prognostic tool, serving as an independent predictor of fatal outcomes.[3] Empiric antibiotic decisions might optimally be guided by the time to positivity (TTP) of blood cultures.[4] TTP serves as a valuable tool for assessing the effectiveness of existing antimicrobial treatments. Additionally, it plays a crucial role in evaluating bacterial load, its growth rate and distinguishing genuine infections from potential contaminants.[2,5] The TTP also serves as the foundation for the application of the "differential TTP method," employed in the diagnosis of catheter-related bloodstream infections.[2]

It's crucial to note that interpreting TTP can be challenging and requires careful analysis. However, TTP is influenced by numerous confounding factors often overlooked in routine practice. These factors encompass blood volume in the bottles, culture conditions (such as atmosphere and broth type), transportation duration, concurrent antimicrobial therapy, and the nature of the sample (venepuncture or catheter-derived).[2] Though there are increasing number of TTP associated articles published over several years, in this study, we have analysed the TTP of different bacterial isolates, and the effects of initiation of antimicrobials and blood volume on TTP.

2. Materials and Methods

2.1. Study setting

This is a single centre retrospective observational study conducted in the Department of Microbiology, a tertiary care hospital in Southern part of India. Blood culture data over a period of 4 years from November 2019 to November 2023 were studied. Adult patients (≥18 years old) with suspected blood stream infections (BSI) who had blood culture positive with pathogens or uncertain pathogens or contaminants were included in the study. The study was conducted as a part of routine investigation. No additional sample was collected as part of project and no active interventions were done on the patient for the purpose of the present study. It is purely a lab-based study which included only samples received in the laboratory for analysis without representing demographic details of the patient during analysis.

2.2. Blood culture processing

Blood cultures obtained from all the adult patients (≥18 years old) with suspected blood stream infections (BSI) were loaded into the automated blood culture system - Bact-Alert Virtuo® (BioMerieux). The various isolates from the positive blood culture bottles were identified by MALDI-TOF MS (VITEK MS®, BioMerieux) and using standardised methodologies. Polymicrobial cultures were excluded from the study as the TTP for individual

pathogens were unknown. Time to positivity was recorded for each positive sample. TTP was taken as the time duration between the loading of the blood culture bottle into the instrument and the positive flagging of the same. Information regarding the timing of blood sample collection i.e. before or after start of antibiotics was collected from the blood culture requisition form. Antibiotic pretreatment was defined as the treatment with one or more antibiotics within 24 hours of blood collection.[6] The blood volume in the blood culture bottle is the virtual blood volume given by the Bact-Alert Virtuo® instrument.

2.3. Analysis

Microbiological data including the pathogen and the time to positivity were retrieved from the data base of the department of Microbiology. Categorical variables were detailed as counts and percentages, whereas continuous variables were described as means and standard deviations or medians and interquartile ranges (IQRs). The blood samples were categorised based on the TTP i.e. blood samples with TTP less than 6 hours, 6-12 hours, 12-24 hours, 24-48 hours, 48-72 hours and more than 72 hours. Effect of timing of blood sample collection i.e. before or after start of antibiotics and blood volume were analysed across various categories of TTP. The proportion of the bacterial cultures based on the TTP were compared in the following groups: 1) Pathogens versus contaminants 2) Gram positive pathogens versus Gram negative ones 3)cultures obtained prior to antibiotic administration. The effect of initiation of empirical antibiotics on TTP was compared using independent t-test. Comparison of the difference in various TTPs with respect to the blood volumes were done using one way ANOVA. Analyses were performed with SPSS software version 29.0.2.0 and the level of statistical significance was set at 5%.

3. Results

During the study period of 4 years, a total of 38,606 blood culture samples that flagged positive from adult patients with suspected BSIs were included. Their TTP distribution has been shown in (Table 1). 79% of the samples had a TTP of less than 24 hours and 15% of the samples had TTP of 24 to 48 hrs. Only 6% of the samples had TTP of more than 48 hours.

Gram negative pathogens outnumbered Gram positive pathogens. Among the Gram negative pathogens, *Escherichia coli* (8.8%) was the most common followed by *Klebsiella pneumoniae* (6.9%). Among the Gram positive pathogens, *Staphylococcus aureus* was the most common one followed by *Enterococcus* species. 15.9% of the bottles had aerobic spore bearers or diphtheroid grown in culture and *Candida* species constituted 3.3% of all the positive blood culture bottles. The proportion, minimum, maximum

Table 1: Blood culture isolate frequency distribution with respect to time-to-positivity

Pathogen/ Pathogen Uncertain / Contaminants	Distribution %(N)
Isolates with TTP <6 h	8% (3134)
Isolates with TTP 6-12 h	28% (11002)
Isolates with TTP 12-24 h	43% (16421)
Isolates with TTP 24-48 h	15% (5972)
Isolates with TTP 48-72 h	4% (1447)
Isolates with TTP >72 h	2% (630)
Total	38606

and average TTPs of various pathogens and contaminants isolates are summarized in (Table 2). Regardless of the number of positive cultures, certain organisms like *Staphylococcus aureus* and *Candida* species are usually predictive of BSIs.[7] The distribution rates of the various organisms across different time periods are summarized in (Table 3).

A total of 33,341 blood samples had data for initiation of the empirical antibiotic therapy i.e. before or after sample collection. Around 78% of the blood samples that flagged positive, sample was collected before the antibiotic was started. There was no significant difference between different TTPs. (Table 4)

A total of 33,109 blood samples that flagged positive had data of blood volume for the culture. Blood volume for culture was less than 8 ml in 46%, 8-10 ml in 40% and more than 10 ml in 13% of the samples. There was no significant difference between different TTPs. Data on blood volumes and its effect on TTP are summarised in (Table 5).

4. Discussion

Blood stream infections (BSI) are one of the serious and life threatening complications associated with high morbidity and mortality, increase in length of hospital stay and cost. Antibiotic consumption especially broad spectrum in the form of prolonged empirical therapy is an important driving force for global emergence of antimicrobial resistance.[8] Upon clinical presentation, the differential diagnosis for any case of pyrexia is often uncertain. It could be either bacterial, viral, thromboembolic events or even severe drug reactions. Identification of patients without bacterial infections is an important component of antimicrobial stewardship. Though there are various biomarkers which could suggest presence of sepsis due to bacterial infections, traditional practice is to wait for the blood culture report for 24-48 hours to differentiate bacteraemia from non-bacteraemic events.[9] However, with the advances in the automated blood culture systems especially with the continuous monitoring systems, time to positivity (TTP) of blood cultures has been reduced drastically thereby allowing faster de-escalation of the antibiotics.[10]

TTP measures the biomass or the load of the organism in the blood. Higher the initial biomass, shorter the TTP and faster detection of the bacterial growth.[11] Faster time to positivity may suggest a higher bacterial load in the blood or faster growth rate of the organism. Conversely, a longer time to positivity may indicate a lower bacterial load or slower-growing organism. In clinical settings, understanding the relationship between TTP and biomass can help clinicians interpret blood culture results more effectively, guiding appropriate treatment decisions for patients suspected of having bloodstream infections. TTP can be used to evaluate the efficacy of the current antimicrobial therapy and an independent predictor of fatal outcome.[3,5,12,13]

The main findings of this study are 1) the great majority of the blood cultures were positive within 12-24 hours 2) Gram negative pathogens have shorter TTP when compared to Gram positive pathogens. In our study, 43% of the blood cultures flagged positive between 12-24 hours followed by 28% between 6-12 hours. Only 2% of the bottles flagged positive beyond 72 hours. This is in consistent with most of the other studies done on adult patients.[6,14–16] Low probability of bacteraemia when the blood cultures remained sterile for more than 72 hours would help in re-evaluation of the patient for alternate causes for infection and subsequently the diagnostic and the therapeutic options. In a study conducted by *Puerta-Alcalde* et al., longer TTP (>24 hours) can be used for earlier antimicrobial de-escalation in febrile neutropenic patients.[17]

4.1. Microbial determinants of time to positivity

TTP can be influenced by different factors like microbial species, bacterial burden, blood volume poured in the blood culture bottles, source of infection and the presence of co-morbidities. Microorganism species is an important factor in influencing the time for bacterial growth detection. In the present study, since we have not excluded Coagulase negative staphylococci (CONS) from the analysis, majority of which could actually be the normal skin flora, Gram positive pathogens outnumbered Gram negative pathogens. Excluding CONS, Gram negative pathogens were the predominant pathogens isolated from the positively flagged bottles. This finding is in consistent to majority of the other studies. Time to positivity can vary between Gram-positive and Gram-negative organisms due to differences in their growth rates, metabolic activity, and other factors. In general, Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus* species[14,18] tend to have longer time to positivity compared to Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*.[12] In this study, time to positivity of different organisms-Gram positive, Gram negative and candida species differed significantly. Gram negative bacilli had lower TTP than Gram positive cocci, Gram positive bacilli and Candida species. 45% of the Gram negative bacilli were flagged

Table 2: TTP of blood culture positive strains. (TTP in hrs)

Microorganisms	Number of strains	Proportion (%)	Average of TTP (hours)	Minimum TTP (hours)	Maximum TTP (hours)
Pathogens					
Gram negative bacilli	12,686	31.5%	12.5	1	**99.6**
Escherichia coli	3523	8.8%	11.1	1.2	98.5
Klebsiella pneumoniae	2771	6.8%	10.5	1.1	98.5
Acinetobacter baumannii	2497	6.2%	10.6	1.2	99.6
Pseudomonas aeruginosa	1413	3.5%	14.7	1.2	99.6
Enterobacter species	583	1.5%	10.7	1.1	97.6
Elizabethkingia species	440	1.1%	13.6	2.7	44.3
Salmonella species	310	0.8%	18.1	3.5	78.3
Aeromonas species	138	0.3%	9.4	1.6	42.8
Other *Enterobacterales*	1011	8%	14.1	1.2	99.4
Gram positive cocci	**4670**	**11.6%**	**15.4**	**1.1**	**100**
Staphylococcus aureus	2248	5.6	14.4	1.2	97.4
Enterococcus species	1466	3.6	16.6	1.2	72.2
Beta hemolytic *streptococci*	435	1.1	13.2	1.2	75.8
Streptococcus pneumoniae	225	0.6	9.5	1.2	48.6
Other Gram-positive cocci	296	0.7	23..4	2.7	92.5
Fungi Candida species	**1308**	**3.25%**	**34.2**	**1.3**	**97.5**
Contaminants	**21,542**				
Coagulase negative staphylococci	11930	29.6%	24.2	1.1	100
Gram Positive Bacilli Diphtheroids & Aerobic spore bearers	6374	15.9%	37.8	1.2	100
Viridans Streptococci	501	1.2%	20.3	2.7	92.5
Other Non ferementing GNRs reported as contaminants	2737	6.8%	29.5	1.8	100
Total no of organisms	**40206**				

between 6-12 hours whereas the 60% Gram positive bacteria had a little longer TTP i.e 12-24 hours. We could not find any significant difference in TTP between different members of the *Enterobacterales* like *Escherichia coli*, *Klebsiella* species. A study by Palmer et al., showed that lactose fermenting GNB have shorter TTP when compared to the non-fermenters.[19] Our study also had similar findings. In another study by Gilles et al., median TTP for bacteraemia due to *Enterobacterales* was 11hrs, 17 hours for *Pseudomonas aeruginosa* and 21 hours for other GNB.[19] *Acinetobacter baumanni* had the shortest TTP among all the Gram negative pathogens in our study. Among the bottles with TTP >24 hours, the predominant pathogen was yeast. This finding underlines the need to optimize microbiological techniques for faster yeast detection.[20]

According to Martinez et al., with respect to the source of the infections, shorter TTP was observed with infections originating from urinary tract or biliary tract.[21] However, in the present study we have not analysed the source of infections in any of the cases of BSIs.

TTP has been used as a prognostic marker for severity of blood stream infections.[15] However, how to use TTP to predict mortality is not clear and requires further research.[3] Several studies has emphasised the fact that short TTP is often associated with increase in the mortality especially in case of *Staphylococcus aureus*[14] and *Escherichia coli*[22] and even a marker for severe sepsis and meningitis in BSI due to *Streptococcus pneumonia*.[18] Interestingly, the relationship between TTP and mortality is not always linear and we should not focus just on the shorter TTP. Longer the TTP, more cautious the treating clinical team be. However, while analysing the TTP as predictor of mortality, lot of variables like the time required to transport the sample to the laboratory and loading of the sample into the machine, volume of the blood taken, mode of collection of the sample- venepuncture or through the catheter, atmosphere and the type of the blood culture broth are not taken into consideration which could actually affect the TTP. In the present study, as soon as the sample was received in the laboratory, it was loaded into the Bact-Alert Virtuo® instrument since we have a 24 x 7 laboratory for the

Table 3: The distribution rates (% n = n/N * 100) of positive isolates across different time periods

Microorganisms Pathogens	<6h TTP	6-12 h TTP	12-24h TTP	24-48h TTP	48-72h TTP	>72h TTP
Gram negative bacteria	27% 3443/12686	51% 6435/12686	22% 2799/12686	3% 393/12686	0.9% 112/12686	0.4% 51/12686
Klebsiella pneumoniae	23% 637/2771	54.6% 1513/2771	18% 502/2771	2.9% 79/2771	1.1% 30/2771	0.4% 10/2771
Escherichia coli	16% 575/3523	62% 2181/3523	16.3% 577/3523	4% 139/3523	0.9% 30/3523	0.6% 21/3523
Pseudomonas aeruginosa	12% 170/1413	26.5% 374/1413	54.4% 768/1413	5.1% 72/1413	1.7% 24/1413	0.4% 5/1413
Acinetobacter baumannii	22% 547/2497	63.5% 1580/2497	12.7% 317/2497	1% 26/2497	0.2% 4/2497	0.04% 1/2497
Elizabethkingia species	11.1% 49/440	37.5% 165/440	50% 211/440	3% 13/440	0.5% 2/440	0% 0/440
Salmonella species	2% 6/310	30% 92/310	60% 185/310	7.4% 23/310	1% 3/310	0.3% 1/310
Aeromonas species	40.5% 56/138	54.3% 75/138	4.3% 6/138	0.7% 1/138	0% 0/138	0% 0/138
Other *Enterobacterales*	27% 272/1011	45% 454/1011	23.1% 234/1011	4% 41/1011	1.5% 15/1011	1.2% 12/1011
Gram positive cocci	11.4% 533/4670	43% 2004/4670	32% 1515/4670	5.2% 243/4670	0.8% 36/4670	0.4% 19/4670
Staphylococcus aureus	11.7% 264/2248	39% 884/2248	38.8% 873/2248	7.9% 177/2248	1.5% 33/2248	0.8% 17/2248
Enterococcus	9% 132/1466	46% 677/1466	39% 577/1466	3.8% 55/1466	1% 14/1466	0.07% 1/1466
Beta hemolytic streptococci	21% 93/437	66% 289/437	10.5% 46/437	1.1% 5/437	0%	0.2% 1/437
Pneumococci	19.5% 44/225	68% 154/225	8.4% 19/225	2.7% 6/225	0.8% 2/225	0%
Fungi Candida species	2.1 % 28/1308	7.9 % 104/1308	37.5 % 490/1308	40 % 524/1308	10 % 136/1308	2% 26/1308
Contaminants						
Viridans streptococci	3.5% 18/501	39% 197/501	41.7% 209/501	10.6% 53/501	1.8% 9/501	0.6% 3/501
Coagulase negative staphylococci	1.3 % 154/11930	11 % 1316/11930	70.6% 8417/11930	14.7% 1759/11930	1.7% 205/11930	0.7%79/11930
Gram Positive Bacilli Diphtheroids & Aerobic spore bearers	4.3% 274/6374	18% 1149/6374	29% 1843/6374	32.5% 2074/6374	10.6% 678/6374	5.6% 356/6374
Other non-fermenting Gram negative bacilli	7.3% 200/2737	16% 460/2737	39% 1091/2737	26.8% 734/2737	7.2% 197/2737	2% 55/2737

Table 4: Effect of initiation of empirical antimicrobials on time-to-positivity of blood cultures

	No. of Samples	Cultures collected % (n/N)	
		Before antibiotic start n% (N)	After antibiotic start n% (N)
Isolates with TTP <6 h	2687	77% (2073/2687)	23% (614/2687)
Isolates with TTP 6-12 h	9529	77% (7348/9529)	23% (2181/9529)
Isolates with TTP 12-24 h	14101	78% (11003/14101)	22%(3098/14101)
Isolates with TTP 24-48 h	5254	78% (4101/5254)	22% (1153/5254)
Isolates with TTP 48-72 h	1244	80% (992/1244)	20% (252/1244)
Isolates with TTP >72 h	526	81% (428/526)	19% (98/526)
Total	33341	78% (25945)	22% (7396)

Table 5: Effect of blood volume on time-to-positivity of blood cultures

	No. of Samples	Blood volume					
		< 8 mL		8-10 mL		≥ 10 mL	
		n%	N	n%	N	n%	N
Isolates with TTP <6 h	2402	47%	1133	41%	990	12%	279
Isolates with TTP 6-12 h	8032	41%	3303	45%	3654	13%	1075
Isolates with TTP 12-24 h	11998	43%	5163	44%	5251	13%	1584
Isolates with TTP 24-48 h	4240	44%	1851	42%	1794	14%	595
Isolates with TTP 48-72 h	662	32%	213	57%	375	11%	74
Isolates with TTP >72 h	201	29%	59	57%	114	14%	28
Total	27535	43%	11722	44%	12178	13%	3635

processing of blood culture samples. However, we could not have a track on the time of collection of the sample and time required to transport the sample to the laboratory. The optimal volume of blood to be collected is a matter of controversy due to the lack of knowledge of the bacterial load as well as the low intravascular volume especially among the children. There was no significant difference in the different TTPs with respect to the volume of the blood collected (P value-0.11, ANOVA single factor).

The strengths of the present study are large number of samples from adult patients with suspected BSIs were included. Most of the other studies were focussed on certain targeted group of patients or pathogens like febrile neutropenia,[17] paediatric patients with sepsis,[23] *Klebsiella pneumonia*,[24] *Pseudomonas aeruginosa* BSIs,[12,13] *Staphylococcus aureus* BSI[25] etc. Our centre offers 24 x 7 microbiological laboratory with continuous processing of all the positively flagged blood culture bottles with the identification of the colony using MALDI-TOF MS. Positive blood culture reports are immediately reported to the treating clinician for further actions. Limitations of the study are its retrospective nature and conducted in a single centre, lack of clinical and microbiological correlation and lack of data on the source of BSIs- catheter related and relationship of drug resistance pattern with TTP. Even with the above mentioned limitations, we would like to conclude that TTP is a useful tool for guidance in the management of the patients. Full implementation of early de-escalation solely based on the TTP may require further research in the following areas- clinical trials based on TTP as a decision marker, multicentre prospective studies for

studying the various confounding variables that can affect the TTP and validation of various cut-off TTPs.

5. Conclusion

With the use of modern automated blood culture systems, TTP can be used as a tool to guide the antimicrobial therapy, early de-escalation of the empirical antibiotics and hence an independent predictor of outcome.

6. Source of Funding

7. Conflicts of Interest

The authors have indicated they have no potential conflicts of interest to disclose.

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